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AUTOLOGOUS BONE MARROW TRANSPLANTATION IN PATIENTS WITH ACUTE NONLYMPHOCYTIC LEUKEMIA, USING EX VIVO MARROW TREATMENT WITH 4-HYDROPEROXYCYCLOPHOSPHAMIDE

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Abstract We studied 25 patients with acute nonlymphocytic leukemia in second remission (20 patients) or third remission (5 patients) in whom autologous bone marrow transplantation was performed with use of marrow incubated ex vivo with the alkylating agent 4-hydroperoxycyclophosphamide. Patients received intensive cytoreductive therapy with busulfan and cyclophosphamide or cyclophosphamide and total body irradiation, followed by an infusion of marrow that had been collected in remission, treated with 4-hydroperoxycyclophosphamide, and cryopreserved.

Four patients died from bacterial or fungal sepsis within the first month after transplantation, and one patient with persistent marrow hypoplasia died from gram-negative sepsis 155 days after infusion with autologous marrow. In the remaining patients, peripheral-blood levels of neutrophils in excess of 0.5×10^9 per liter and platelet counts over 50×10^9 per liter were attained at median intervals of 29 and 57 days after transplantation, respectively. Nine patients had leukemic relapses at 73 to 316 days (median, 182 days) after infusion of autologous marrow, for an actuarial relapse rate of 46 percent. Eleven patients (eight in second remission and three in third) remained in remission at a median of more than 400 days (range, >230 to >1653 days) after transplantation.

The observed disease-free survival after transplantation with autologous marrow treated with 4-hydroperoxycyclophosphamide compares favorably with the results of syngeneic or allogeneic transplantation in similar groups of patients. (N Engl J Med 1986; 315:141-7.)

CURRENT intensive regimens of chemotherapy for the treatment of acute nonlymphocytic leukemia result in prolonged first remissions in a substantial proportion of patients, who may ultimately be cured of their leukemia.[1-3] However, patients who have one or more hematologic relapses have an extremely poor prognosis and a minimal chance of leukemia-free survival. Allogeneic bone marrow transplantation in such patients provides an opportunity for cure of leukemia, although graft-versus-host disease and opportunistic infections — notably viral interstitial pneumonitis — account for most of the mortality after allogeneic transplantation.[4-7] Furthermore, 60 to 75 percent of the patients who might benefit from allogeneic marrow transplants for leukemia lack related HLA-matched donors.

Transplantation with autologous marrow permits the administration of intensive myeloablative antileukemic therapy followed by the infusion of the patient's own marrow, previously collected during remission and cryopreserved. In patients with acute nonlymphocytic leukemia in second or subsequent remission, the use of autologous-marrow "rescue" is limited by the possibility that marrow samples obtained during remission may contain residual viable leukemia cells. Preclinical studies have shown that leukemia cells in rodents are eliminated from marrow suspensions by ex vivo incubation with 4-hydroperoxycyclophosphamide,[8] a congener of cyclophosphamide and an active alkylating agent in aqueous solution.[9] Recently, a Phase I trial of ex vivo treatment of marrow with 4-hydroperoxycyclophosphamide and autologous transplantation in patients with leukemia and lymphoma demonstrated that marrow can be incubated with up to 100 μg of 4-hydroperoxycyclophosphamide per milliliter without impairment of the normal hematopoietic repopulating ability.[10]

In this Phase II study we examined the therapeutic efficacy of autologous-marrow transplantation in 25 patients with acute nonlymphocytic leukemia in second or third remission, using marrow incubated ex

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vivo with 60 to 100 μg of 4-hydroperoxycyclophosphamide per milliliter. We studied the acute toxic effects of the preparative regimens, infectious complications, rates of hematologic reconstitution, and durations of survival and leukemia-free survival after autologous transplantation.

Methods

Informed Consent

All protocols for ex vivo marrow treatment and intensive myeloablative therapy before transplantation were reviewed and approved by the Joint Committee on Clinical Investigation of the Johns Hopkins Medical Institutions. Informed consent was obtained from all patients and, in the case of children, from their parents.

Patients

Twenty-five consecutive patients (10 male and 15 female) were included in this study (Table 1). Their median age was 31 years (range, 4 to 53). The diagnosis of acute nonlymphocytic leukemia was confirmed in all the patients by examination of bone-marrow aspirates, and histologic types of leukemia were classified according to the system of the French–American–British (FAB) Cooperative Group.[11] All the patients had had at least one documented hematologic relapse of leukemia and had received intensive chemotherapy to induce the initial remission and subsequent remissions. The median duration of the first remission was 18 months (range, 2 to 96). At the time of marrow collection and transplantation, 20 patients were in their second remission and 5 were in their third. One patient (unique patient number [UPN] 456) had a history of leukemic involvement of the central nervous system at the time of diagnosis, and another (UPN 595) had both central nervous system and testicular leukemic involvement at the time of first relapse. No patients had active extramedullary leukemia at the time of autologous-marrow transplantation. Three patients in this series (UPNs 258, 309, and 314) had been described previously in the dose-escalation study of 4-hydroperoxycyclophosphamide.[10]

Collection, Processing, and Infusion of Marrow

Patients underwent marrow collection a median interval of 2 months (range, 0.5 to 5) after entering their second or third remission (Table 2). Marrow was collected from the posterior iliac crests according to the methods of Thomas and Storb.[12] For each patient, a collection of 4 to 6×10^8 nucleated marrow cells per kilogram of body weight was attempted. Approximately 70 percent of the collected marrow was treated ex vivo with 4-hydroperoxycyclophosphamide at a concentration of 60 μg per milliliter in one case, 80 μg per milliliter in two cases, and 100 μg per milliliter in the remaining 22 cases. The mean number (±1 SE) of nucleated marrow cells treated with these doses of 4-hydroperoxycyclophosphamide was $3.1\pm0.17\times10^8$ per kilogram (range, 1.6 to 5.3×10^8). In 23 patients, the remainder of the collected marrow-cell suspension was treated with a lower dose of 4-hydroperoxycyclophosphamide (40 or 60 μg per milliliter) or with no drug, and was reserved for infusion in the event that engraftment with the fully treated autologous-marrow inoculum failed. Because of suboptimal collections, two patients (UPNs 574 and 583) had no reserve marrow available. The nucleated-cell buffy coat of the marrow was removed from the blood–bone marrow suspension by centrifugation in standard blood-transfer packs (Fenwal) in a Sorvall RC-3B centrifuge with an HG-4L head at 2900 rpm for 10 minutes, and the centrifugation process was repeated to extract a second buffy-coat layer. The nucleated-cell

Table 1. Characteristics of Patients Undergoing Autologous Bone Marrow Transplantation for Acute Nonlymphocytic Leukemia.*

Unique Patient No.	Age/Sex	Remission Status at BMT	FAB Class	Duration of 1st/2nd Remission	Previous Therapy	Maintenance Chemotherapy	Time between Remission and Marrow Collection
				mo			mo
258	6/F	CR2	M2	10	AraC, DNR, ADR, TG, VCR, PR, DEX	Yes	2
309	45/M	CR3	M1	18/3	AraC, DNR, ADR, TG, VCR, PR	Yes	3
314	31/M	CR2	M1	17	AraC, DNR, TG, VCR, PR	Yes	2
344	19/M	CR3	M4	17/12	AraC, VCR, TG, PR, DEX, CY, AMSA, VP16	Yes	1.5
405	39/M	CR2	M2	9	AraC, DNR, TG, AMSA, AZA	Yes	0.5
416	4/F	CR2	M1	20	AraC, DNR, TG, VCR, PR, CY	Yes	1.5
423	12/F	CR2	M1	12	AraC, DNR, TG, VP16, AZA	Yes	3
425	36/F	CR2	M2	15	AraC, DNR, ADR, TG	No	2
431	15/F	CR2	M1	14	AraC, DNR, VCR, PR, CY, L-ASP, AZA	Yes	1
456	22/F	CR2	M4	31	AraC, DNR	Yes	2
473	21/F	CR2	M5	30	AraC, DNR, TG	Yes	4
524	35/F	CR2	M4	41	AraC, DNR, TG, VCR, PR	Yes	5
543	21/F	CR2	M1	23	AraC, DNR, TG, VCR, PR, DEX	Yes	1.5
574	42/F	CR2	M4	17	AraC, DNR, TG, AMSA, AZA, MTX	No	4
582	32/F	CR3	M3	19/25	AraC, DNR, PR, CY, AMSA, VP16	No	2
583	25/M	CR3	M1	30/18	AraC, DNR, ADR, TG, VCR, PR, RUB	Yes	2
584	53/F	CR2	M4	19	AraC, DNR, ADR, TG	Yes	4
595	38/M	CR3	M5	2/5	AraC, DNR	No	2
596	20/M	CR2	M4	4	AraC, DNR, TG, AMSA	No	1
598	7/M	CR2	M2	37	AraC, DNR, VCR, PR, CY, AZA	Yes	3
603	39/F	CR2	M1	53	AraC, DNR	Yes	2
606	41/F	CR2	M1	12	AraC, DNR, TG	Yes	2
616	40/M	CR2	M1	96	AraC, DNR, TG, VCR, DEX	Yes	3.5
619	52/M	CR2	M4	12	AraC, DNR, AMSA	Yes	2.5
629	5/F	CR2	M3	19	AraC, DNR, CY, VP16, L-ASP	Yes	2

*BMT denotes bone marrow transplantation, FAB French–American–British Co-operative Group, CR complete hematologic remission, AraC cytarabine hydrochloride, DNR daunorubicin, ADR doxorubicin hydrochloride, TG thioguanine, VCR vincristine, PR prednisone, DEX dexamethasone, CY cyclophosphamide, AMSA amsacrine, VP16 etoposide, L-ASP L-asparaginase, AZA 5-azacitidine, MTX methotrexate, and RUB rubidazone.

fractions thus obtained were mixed with autologous plasma and heparinized tissue-culture medium (TC 199; Gibco) to obtain a concentration of 2×10^7 cells per milliliter. An appropriate volume of a freshly prepared solution of 4-hydroperoxycyclophosphamide in phosphate-buffered saline was added to the cell suspension to obtain the desired final concentration of drug. Cells were incubated with 4-hydroperoxycyclophosphamide at 37°C for 30 minutes, after which the cell suspension was rapidly cooled to 4°C and centrifuged at 2900 rpm for 10 minutes. The drug-treated cells were resuspended in 45 percent TC 199, 45 percent autologous plasma, and 10 percent dimethylsulfoxide at a concentration of 4×10^7 cells per milliliter, and 50-ml aliquots of the cell suspension were placed in polyolefin bags (Del-Med). The bags were frozen in a controlled-rate freezer (Cryo-Med) at −1°C per minute to a temperature of −50°C and at −10°C per minute to a temperature of −70°C, and then transferred to the liquid phase of a liquid-nitrogen freezer. At the time of autologous-marrow rescue (designated as day 0), each bag was thawed rapidly in a 37°C water bath, and the thawed cell suspension was infused through a large-bore central venous catheter at a rate of 10 to 15 ml per minute.

Hematopoietic Progenitor-Cell Assays

For each patient, samples of marrow treated with 4-hydroperoxycyclophosphamide were obtained for assays of granulocyte–macrophage colony-forming cells in a soft-agar culture system, as previously described.[10] Mononuclear cells from the marrow were cultured in 35-mm plastic tissue-culture dishes (Falcon) containing 0.3 percent agar, 15 percent fetal bovine serum, and McCoy's 5A medium, with human placental-conditioned medium as the source of colony-stimulating factor. After incubation at 37°C in 7.5 percent carbon dioxide in humidified air for 10 to 12 days, culture dishes were examined at a magnification of 35 to 40 for the presence of colonies of granulocytes and macrophages; aggregates of more than 40 cells were considered colonies. The total number of granulocyte–macrophage colony-forming cells infused into each patient was calculated from the dose of nucleated marrow cells infused and the frequency of colonies of granulocytes and macrophages in culture.

Preparative Regimens

The preparative regimens employed in these patients were identical to those used for allogeneic-marrow transplantation at this institution.[7,13] Twenty-three patients received busulfan (1 mg per kilogram of body weight per dose orally every six hours for 16 doses) on days −9, −8, −7, and −6, followed by cyclophosphamide (50 mg per kilogram per dose intravenously) on days −5, −4, −3, and −2; autologous marrow was infused 48 hours after the last dose of cyclophosphamide. Two patients with a history of leukemic involvement of the central nervous system received cyclophosphamide (50 mg per kilogram per dose intravenously) on days −8, −7, −6, and −5, and total body irradiation (300 rad per day, with the lungs shielded after 900 rad) on days −4, −3, −2, and −1, with autologous-marrow rescue 24 hours after the last dose of irradiation. Prophylactic consolidation chemotherapy was initiated 60 to

Table 2. Hematologic Reconstitution and Clinical Status after Autologous Bone Marrow Transplantation for Acute Nonlymphocytic Leukemia.*

Unique Patient No.	Dose of 4HC	No. of Cells Infused		Time to Attain		Relapse	Survival	Status
		NBMC	GM-CFC	ANC >0.5×10⁹/liter	Platelets >50×10⁹/liter			
	μg/ml	(×10⁸/kg)	(×10³/kg)	days after transplantation				
258	60	5.0	0	21	34	165	>1909	Alive (relapsed, on therapy)
309	80	2.8	5.0	16	70	—	>1653	Alive, in remission
314	80	2.3	0	20	63	194	677	Dead (leukemia)
344	100	2.6	0	24	47	182	263	Dead (leukemia)
405	100	3.4	5.1	63	>162	162	218	Dead (leukemia)
416	100	5.3	0	31	35	213	623	Dead (leukemia)
423	100	3.6	0	44	>73	73	166	Dead (leukemia)
425	100	3.7	0.19	24	84	—	>1073	Alive, in remission
431	100	2.5	0	NE	NE	NE	24	Dead (*Str. viridans* sepsis, meningitis)
456	100	2.6	0	35	50	—	>933	Alive, in remission
473	100	2.6	0.39	28	50	—	>875	Alive, in remission
524	100	2.9	0	27	45	—	>664	Alive, in remission
543	100	3.9	1.17	37	37	157	161	Dead (leukemia)
574	100	2.2	1.32	NE	NE	NE	155	Dead (sepsis, aplasia)
582	100	3.2	0.96	27	191	—	>400	Alive, in remission
583	100	1.6	0.32	29	36	316	>399	Alive (relapsed, on therapy)
584	100	3.0	0	NE	NE	NE	12	Dead (*Pseud. aeruginosa* sepsis)
595	100	4.2	0	42	73	—	>355	Alive, in remission
596	100	3.2	1.76	42	132	—	>355	Alive, in remission
598	100	3.2	0	20	23	—	>348	Alive, in remission
603	100	3.1	0.62	29	64	245	266	Dead (leukemia)
606	100	2.5	1.75	27	>307	—	>307	Alive, in remission
616	100	2.8	0	NE	NE	NE	17	Dead (*Pseud. aeruginosa* sepsis)
619	100	2.5	0	NE	NE	NE	9	Dead (*Candida tropicalis* sepsis)
629	100	3.6	0	46	43	—	>230	Alive, in remission

*4HC denotes 4-hydroperoxycyclophosphamide, NBMC nucleated bone marrow cells, GM-CFC granulocyte–macrophage colony-forming cells in culture, ANC absolute neutrophil count, BMT bone marrow transplantation, and NE not evaluated.

70 days after marrow transplantation and consisted of five doses of intrathecal methotrexate (10 mg per square meter of body-surface area; maximal dose, 12 mg), administered twice weekly over a period of 2½ to 3 weeks. Patients with a history of leukemia involving the central nervous system also received monthly intrathecal injections of methotrexate for a total of 12 additional doses.

Supportive and Post-transplantation Care

Patients were nursed in single rooms with high-efficiency particulate air-filtration systems that provided 32 nonlaminar air exchanges per hour. Before marrow transplantation, one or two indwelling central venous catheters were placed in all patients[14] for the administration of fluids, antibiotics, and blood products. All patients at risk for the recurrence of herpes simplex infection (manifested by an IgG antibody titer of ≥1:8) received prophylactic intravenous acyclovir.[15] When absolute neutrophil counts fell below 0.5×10^9 per liter, reverse isolation with the use of masks and good handwashing practices was employed. Broad-spectrum antibiotics were given for fever during aplasia, and amphotericin B was added for documented systemic fungal infections or for persistent fever during aplasia. Antibiotics and isolation procedures were discontinued when the patients were afebrile and when absolute neutrophil counts consistently exceeded 0.5×10^9 per liter. All blood products were irradiated with 1500 to 3000 rad before infusion, to prevent possible graft-versus-host reactions.

Statistical Analysis

Routine statistical calculations were performed with a hand-held calculator (Texas Instruments). Survival analysis was performed according to the methods of Kaplan and Meier,[16] with use of a microcomputer (International Business Machines) and statistical software packages developed by the Biostatistics and Information Systems Division of the Oncology Center of the Johns Hopkins University School of Medicine.

Results

Post-transplantation Clinical Course

Most patients receiving busulfan and cyclophosphamide had moderate oral mucositis, which responded to good oral hygiene but which on occasion required the topical application of agents such as lidocaine and diphenhydramine. Mucositis generally resolved 10 to 14 days after transplantation. No episodes of severe life-threatening hemorrhage were observed. All the patients had fevers during aplasia. In 14 of the 25 patients (56 percent), these fevers were associated with positive blood cultures for bacteria (12 patients) or fungus (2 patients). Three patients died from overwhelming infections during aplasia, 9 to 17 days after transplantation: two with sepsis from *Pseudomonas aeruginosa* and one with sepsis from *Candida tropicalis*. One patient died with meningitis and sepsis due to *Streptococcus viridans* 24 days after the infusion of autologous marrow, despite early engraftment and recovery of peripheral leukocyte counts. One patient (UPN 574) had persistent marrow hypoplasia and died from gram-negative sepsis 155 days after marrow transplantation. In the other 20 patients, no episodes of late systemic bacterial or fungal infection were observed. Nonfatal interstitial pneumonitis due to *Pneumocystis carinii* occurred 130 days after marrow infusion in one patient (UPN 543) who had not been receiving prophylactic trimethoprim–sulfamethoxazole. There was no interstitial pneumonitis attributable to viral agents such as cytomegalovirus.

Engraftment and Hematologic Reconstitution

After incubation with 4-hydroperoxycyclophosphamide, marrow samples from most of the patients had no detectable granulocyte–macrophage colony-forming cells, although in five cases more than 1.0×10^3 colony-forming cells per kilogram were present in the infused marrow (Table 2). There was no correlation between the number of nucleated marrow cells or granulocyte–macrophage colony-forming cells infused and the rate of hematologic recovery. Twenty-one patients could be evaluated for engraftment (the four patients who died during aplasia or early hematopoietic recovery were excluded from this analysis). Sepsis with *Klebsiella pneumoniae* developed in one patient with leukemia in second remission (UPN 574) one day before transplantation, and the patient was hemodynamically unstable at the time of marrow infusion. She recovered from that episode of sepsis but had persistently low leukocyte and neutrophil levels after autologous transplantation (total leukocytes, $<0.4\times10^9$ per liter, absolute neutrophils, $<0.1\times10^9$ per liter, and platelets, $<50\times10^9$ per liter). No reserve marrow was available for infusion in this patient after engraftment with drug-treated marrow failed, and she died from bacterial sepsis during persistent aplasia. Hematologic reconstitution occurred in the 20 other patients, none of whom required infusion of reserve autologous marrow. The median time required to attain an absolute neutrophil count in excess of 0.5×10^9 per liter was 29 days (range, 16 to 63) after transplantation. Two patients (UPNs 405 and 423) had recovery of leukocyte counts but had persistent thrombocytopenia at the time of leukemic relapses 162 and 73 days after transplantation, respectively. One patient (UPN 606) remains in remission with thrombocytopenia (platelet counts, 30 to 35×10^9 per liter) at this writing, 307 days after autologous-marrow rescue. In the remaining 17 patients, the median time required to attain a platelet count exceeding 50×10^9 per liter was 57 days (range, 23 to 191) after transplantation.

Leukemic Relapses and Disease-Free Survival (Table 2)

Among the 20 evaluable patients, hematologic leukemic relapses occurred in 7 of 15 patients who received transplants during their second remission and in 2 of 5 patients who received transplants during their third remission, a median interval of 182 days (range, 73 to 316) after autologous-marrow rescue, for an actuarial relapse rate of 46 percent. No nervous system or gonadal involvement with leukemia was apparent at the time of relapse. The relapses occurred in six patients whose disease had been assigned to FAB class M1, two patients with disease of class M2, and one patient with disease of class M4. There was no significant difference in the duration of first

remission between patients who relapsed and those who were disease free ($P = 0.1092$ by Wilcoxon rank-sum test).

Similarly, there was no difference between these two groups in the length of time between the start of remission and marrow transplantation ($P = 0.0878$). Reinduction therapy was successful in inducing a prolonged remission in only one of these nine patients; seven patients died from recurrent leukemia or from complications related to its treatment. Eleven patients (8 of 15 in second remission and 3 of 5 in third remission) remain at this writing in unmaintained hematologic remissions after autologous transplantation, with a median duration of leukemia-free survival of more than 400 days (range, >230 to >1653). In five patients (UPNs 309, 425, 456, 595, and 596), the duration of second or third remission after autologous-marrow transplantation exceeds the duration of their first remission. Actuarial survival analysis[16] indicates a 43 percent probability of overall survival and disease-free survival (Fig. 1).

Discussion

Intensive chemoradiotherapy with infusion of histocompatible marrow is curative in many patients with acute leukemia. The use of autologous marrow obtained during remission to provide hematopoietic stem cells may have certain theoretical and practical advantages over the use of allogeneic marrow. Although the absence of graft-versus-host disease after autologous-marrow transplantation eliminates one of the major causes of death in recipients of allogeneic transplants, one would anticipate that the risk that leukemia will recur is greater after autologous-marrow rescue than after allogeneic transplantation. This speculation is supported by the high relapse rate (50 percent) observed in recipients of syngeneic marrow transplants,[17] a phenomenon attributable at least in part to the absence of allogeneic graft-versus-leukemia effects.[18,19] The disease-free survival after autologous-marrow transplantation must be analyzed in relation to survival data obtained after syngeneic transplantation in comparable groups of patients — i.e., in clinical situations in which tumor cells are absent from the marrow inoculum and allogeneic graft-versus-host and graft-versus-leukemia reactions do not occur.

Autologous-marrow transplantation for acute leukemia in second or subsequent remission presents two problems: eradication of residual leukemia in vivo and elimination of clonogenic tumor from the marrow-cell suspension ex vivo. Attempts to remove occult leukemic cells from autologous marrow by such separation methods as density-gradient centrifugation have been unsuccessful.[20] Although preliminary results of the ex vivo treatment of autologous marrow with immunologic purging techniques are encouraging in acute lymphocytic leukemia,[21,22] the lack of satisfactory monoclonal antibodies that react with myeloid blast cells currently limits the applicability of such immunotherapeutic techniques to acute nonlymphocytic leukemia.

Studies with a transplantable murine lymphoma[23] first demonstrated the difference in sensitivity between neoplastic and normal hematopoietic cells to selected antitumor agents, suggesting that incubation of marrow suspensions with pharmacologic agents might effectively eradicate ("purge") residual leukemic cells while sparing normal hematopoietic stem cells. Phase I clinical studies of dose escalation using marrow treated ex vivo with the alkylating agent 4-hydroperoxycyclophosphamide[9] in patients with leukemia and lymphoma have demonstrated that hematologic recovery occurs with reasonable promptness after the infusion of autologous marrow incubated with up to 100 μg of 4-hydroperoxycyclophosphamide per milliliter. Persistent aplasia occurred in three of seven patients receiving marrows incubated with 120 μg of the drug per milliliter, suggesting that that concentration was unacceptably toxic to normal human hematopoietic stem cells.[10]

Incubation of human marrow cells with 4-hydroperoxycyclophosphamide greatly reduces the frequency of committed[10,24] and multilineage[24] hematopoietic progenitor cells in culture systems in vitro, although more primitive normal hematopoietic blast cells in culture may be less sensitive to the drug.[25] With one exception, incubation of autologous marrow with 60 to 100 μg of 4-hydroperoxycyclophosphamide per milliliter did not prevent hematopoietic reconstitution after myeloablative therapy in the patients described here, despite the virtual absence of granulocyte–macrophage colony-forming cells in the infused marrows. The recovery of neutrophil and platelet levels was nevertheless delayed for several weeks after marrow infusion, in a manner similar to that observed in the reconstitution of cells in peripheral blood after autologous

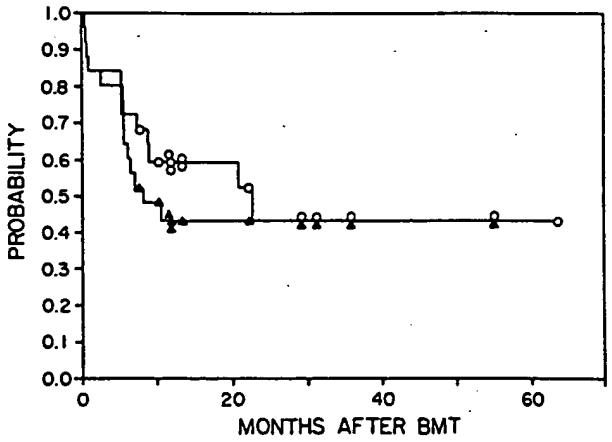

Figure 1. Survival Analysis in 25 Patients Undergoing Transplantation with Autologous Bone Marrow (BMT) for Acute Nonlymphocytic Leukemia in Second Remission (20 Patients) or Third Remission (5 Patients).

Overall survival (open circles) and disease-free survival (solid triangles) are shown; individual living patients are indicated by the symbols.

transplantation for acute leukemia with untreated marrow[26,27] or marrow incubated with ASTA Z 7557, an oxazophosphorine derivative of 4-hydroperoxycyclophosphamide.[28-30] Despite prolonged thrombocytopenia, life-threatening hemorrhages did not occur, and most patients did not require platelet transfusions for more than four to five weeks after transplantation, by which time platelet counts exceeded 20×10^9 per liter but remained below 50×10^9 per liter. Of interest is the observation that four patients have had persistent thrombocytopenia (platelet levels $<50\times10^9$ per liter) for 4 to more than 10 months after the infusion of autologous drug-treated marrow. A similar finding has been reported by Burnett et al.[27] in a group of patients receiving unpurged marrow autografts for acute nonlymphocytic leukemia in first remission. Whether these patients have had damage to the megakaryocytopoietic stem-cell population or have a poorly compensated thrombocytolytic state after autologous-marrow rescue is not known. On balance, the hematopoietic toxicity observed after transplantation of autologous marrow treated with 4-hydroperoxycyclophosphamide appears acceptable and is similar to that observed with intensive regimens for the induction of remission in acute leukemia.[1-3]

Five of the 25 patients in this series (20 percent) died from overwhelming bacterial or fungal infection during aplasia or early hematologic recovery; the frequency of fatal sepsis after allogeneic[7,31] (and Santos GW: unpublished data) or syngeneic[17] marrow transplantation for acute nonlymphocytic leukemia is 5 percent or less. However, additional factors must be considered in the analysis of deaths related to infection in our patients. Two early deaths from sepsis due to *Pseud. aeruginosa* were clustered temporally and were due to a strain of that organism resistant to multiple drugs, and the late death from gram-negative sepsis occurred in the context of persistent neutropenia and marrow hypoplasia. In contrast, no patients with autologous transplants contracted viral interstitial pneumonitis, which is a frequent and often fatal complication of allogeneic transplantation[4-7] but which has a much lower incidence in recipients of syngeneic transplants.[32] The one case of interstitial pneumonitis due to *P. carinii* developed in the absence of prophylactic therapy with trimethoprim–sulfamethoxazole and most likely would have been prevented by the administration of the drug.

Intensive chemoradiotherapy and autologous transplantation with marrow incubated ex vivo with 4-hydroperoxycyclophosphamide may provide long-term control of leukemia in patients with acute nonlymphocytic leukemia in second or third remission, in whom the conventional therapy currently available is not curative. The actuarial rate of relapse (46 percent) after autologous-marrow transplantation is at least comparable to that observed after syngeneic marrow transplantation,[17] and the disease-free survival (43 percent) is similar to that obtained with allogeneic transplantation.[6,7,31,33] Current techniques do not allow one to determine whether the recurrence of leukemia after autologous-marrow transplantation is due to the failure of the intensive preparative regimen to destroy residual leukemia in vivo or to the incomplete eradication of viable tumor cells by ex vivo marrow treatment. More intensive pretransplantation chemotherapeutic regimens may be required to eliminate a greater proportion of residual tumor cells in vivo and to offset the loss of allogeneic graft-versus-leukemia effects. In addition, the development of strategies to treat marrow ex vivo with single-agent and multiple-agent chemotherapeutic or combined immunopharmacologic methods may provide more effective eradication of viable tumor cells from autologous-marrow suspensions in patients with acute nonlymphocytic leukemia.

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IN SEARCH OF THE SUBCUTANEOUS-INSULIN-RESISTANCE SYNDROME

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Abstract In numerous patients with diabetes mellitus, a defect in the absorption of subcutaneously injected insulin has been suspected as an explanation for diabetic instability. The common clinical characteristic of these patients is poor metabolic control when insulin is injected subcutaneously, but good metabolic control when the insulin is infused intravenously.

We have used three approaches to attempt to identify patients with "subcutaneous-insulin resistance." First, we performed a series of studies of subcutaneous-insulin absorption in 16 patients referred to us with a presumptive diagnosis of resistance to subcutaneous insulin; in none of these patients did we detect an abnormal response of blood glucose levels to insulin administered subcutaneously. Plasma free-insulin levels rose normally after injection. Second, we assayed insulin-degrading activity in subcutaneous biopsy specimens obtained from 25 patients throughout North America and Europe who had been diagnosed as resistant to subcutaneous insulin. In none of these patients did the insulin-degrading activity of subcutaneous tissue exceed the mean value (±2 SD) of eight subcutaneous biopsy specimens obtained from control patients with diabetes. Third, we performed studies of tritiated-insulin absorption in three additional diabetic patients and three control patients with nonbrittle diabetes. These studies also suggested normal absorption of insulin.

In none of the patients we studied were we able to confirm the clinical diagnosis of subcutaneous-insulin resistance. We therefore conclude that this syndrome is extremely rare and that misdiagnosis is common. (N Engl J Med 1986; 315:147-53.)

POOR control of blood glucose concentrations in insulin-dependent diabetic patients can be attributed to many variables, including dietary indiscretion,[1] inappropriate schedules of insulin injection,[2] an absence of endogenous insulin,[3] and an inconsistency in the rate of insulin absorption.[4] In some patients, no apparent cause can be found.[5] In 1979, a patient with poorly controlled diabetes was described in whom insulin was rapidly degraded in the subcutaneous tissue by an insulin-specific protease.[6] After several months of intravenous insulin therapy, the patient's disease spontaneously went into remission for five years; the patient then died of unrelated complications of diabetes. This patient's insulin resistance had a precedent in animal studies, in which insulin had been shown to be degraded by subcutaneous tissue.[7,8]

Since the first description of a patient with insulin resistance secondary to excessive subcutaneous-insulin-degrading activity,[6] many additional patients with diabetes have been described as having poor metabolic control caused by this or a similar mechanism.[9-20]

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